Conformations of the Nucleoside Analogs Formycin, 2-Azaadenosine, and Nebularine in Solution

H.-D. Lüdemann and E. Westhof

Institut für Biophysik und physikalische Biochemie, Universität Regensburg

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Formycin, Nebularine, 2-Azaadenosine, S $\rightleftarrows$ N Equilibrium

The ribose conformations of formycin, 2-azaadenosine, nebularine, 8-bromoinosine, and 8-bromoxanthosine have been studied using proton magnetic resonance in ND_3 solutions, in D_2O solutions, and in pyridine solutions. The temperature was varied between -60 °C and $+40$ °C in ND_3 and between $+10$ °C and $+60$ °C in D_2O solutions. In solution, 2-azaadenosine and nebularine have a conformational behaviour similar to that of the common purine(β)ribosides. This is in agreement with the conformations observed in the solid state. The conformations of formycin and formycin B have strong analogies with those of the 8-bromopurineribosides and differ significantly from those of the 8-azapurineribosides since they adopt preferentially the *syn*-S-g^+ conformation. This conformation is very probably stabilized by an intramolecular hydrogen bond between O(5′) and N(3).

1. Introduction

Nucleoside analogs have proved to be of great value as therapeutic agents[1, 2]. Indeed, as stated by Bloch, "there is probably no other class of compounds wherein relatively small structural changes lead to such a variety of different biological effects"[3]. In order to understand their therapeutic properties, one needs correlations between structural changes and biological activity. It is, however, extremely difficult to determine the mode of action of a given nucleoside. Indeed, nucleosides undergo extensive metabolic conversion, thereby affecting a variety of cell constituents. The knowledge of the preferred conformations adopted by nucleoside analogs in solution may help to elucidate their primary mechanisms of action. For example, a nucleoside analog presenting conformations similar to those of the common nucleosides would be incorporated in DNA or RNA, interfering thus with the cell growth. On the other hand, a nucleoside analog presenting unusual conformations is not expected to be incorporated normally in nucleic acids, thereby inhibiting cell activity at another stage.

The naturally occurring and the synthetic nucleoside analogs result from structural modifications in the heterocycle as well as in the carbohydrate moiety. In this paper, we are only concerned with the base modifications. The base can be modified in the substituent, *e. g.* nebularine, N6-isopentenyladenosine. Several base modifications are introduced by the interchange of carbon and nitrogen. These can consist of the rearrangement of the C and N atoms of the base ring, *e. g.* formycin; of the replacement of a base carbon atom with a nitrogen, *e. g.* 8-azaadenosine; or of the replacement of a base nitrogen atom with carbon, *e. g.* tubercidin. Changes in the size of the heterocycle lead also to biological activity. In previous papers[4, 5], we have already analysed the conformational changes introduced by substituents at C(2) or C(6) and by interchange of carbon and nitrogen (8-azapurinenucleosides). We now analyse the conformations of formycin, 2-azaadenosine, and nebularine in ND_3 and D_2O solutions as well as of 8-bromoinosine and 8-bromoxanthosine. We consider also the differences and similarities between the conformations observed in the solid state and in solution.

2. Experimental

2-Azaadenosine (2aA) was kindly supplied by Dr. Montgomery from the Southern Research Institute (Birmingham, Alabama) and formycin (Fo) by Dr. Umezawa from the Institute of Microbial Chemistry (Tokyo). 8-Bromoxanthosine (8-BrX) was a gift from PWA AG (Mannheim, BRD). 8-Bromoinosine (8-BrI) and nebularine (PR) were purchased from the same company. Formycin B (FoB) was purchased from Calbiochem (Lucerne, Switzerland). Deuteriumoxide ("100%" deuterated) and trideuteroammonia (99% deuterated) were bought from Sharp and Dohme Ltd. (München,

Requests for reprints should be sent to Priv. Doz. Dr. H.-D. Lüdemann, Institut für Biophysik und Physikalische Biochemie der Universität Regensburg, Postfach 397, *D-8400 Regensburg.*

BRD). The trideuteroammonia solutions are kept in sealed thickwalled 5 mm tubes. Details for the preparation of these sample tubes were given earlier[4, 6]. The concentration of these solutions is 0.12 molal, except for 2aA where approximately $2\ \mathrm{mg \cdot cm^{-3}}$ were used. The aqueous solutions of Fo, FoB, PR, and 8-BrI are 0.03 molal.

The chemical shifts of the aqueous solutions were referenced to internal DSS (sodium-2,2-dimethyl-2-silapentanesulfonate). For technical reasons, the solutions in liquid ammonia were referenced to an external standard (2% TMS dissolved in CS_2) contained in a coaxial outer tube. No attempts were made to correct for bulk magnetic susceptibility effects. The rather large differences observed between the chemical shifts of the various protons in water and in liquid ammonia may partly be due to the influence of the magnetic susceptibility and its temperature dependence. However, only the relative differences of the resonance frequencies within each set of coupled protons enter into the calculation of the simulated spectra, and these differences are determined by the accuracy of the Fourier-transform spectra (± 0.1 Hz) the digital resolution of the spectra being 0.028 Hz.

The PMR spectra were obtained in the FT-mode on a Varian XL-100-15-FT spectrometer equipped with a 16 K 620/L-100-computer. The temperatures given are accurate to $\pm 0.5\ ^\circ\mathrm{C}$. They were determined with a calibrated chromel alumel thermocouple inserted into a 5 mm NMR tube and set into the probe volume before and after each experiment which lasted between 5 and 20 min. During the experiments the spectrometer was locked either to the deuteron resonance of the solvent or to an external ^{19}F lock. The sugar proton spectra were analysed with the help of the computer program LAME (QCPE N° 111).

The simulation of the spectra was considered successful if the deviations between all observable lines in the experimental and simulated spectra were $\leq \pm 0.1$ Hz. The values for the chemical shifts given in the tables were rounded off to ± 0.01 ppm (~ 1.0 Hz). The original results obtained from the computer analysis together with the values found at intermediate temperatures ($0\ ^\circ\mathrm{C}$ and $-30\ ^\circ\mathrm{C}$ in ammonia and $+40\ ^\circ\mathrm{C}$ in heavy water) have been omitted from the paper, but are available upon request.

The intramolecular distances between O5′ and N3 were calculated from solid state data by application of the program COORD (QCPE N° 136). The dihedral angles for the N and S states of the ribose are taken from Altona and Sundaralingam[7].

3. Conformational Analysis

The three major modes of internal motion of nucleosides are represented in Fig. 1:

(i) pseudorotation of the ribose[7, 8] considered as an equilibrium between the two states S and N: $S \rightleftarrows N$;

(ii) rotation of the hydroxymethyl group[9] around the C(4′)-C(5′) bond analysed in terms of the three classical rotamers: *gauche*$^+$, *trans*, *gauche*$^-$;

(iii) rotation of the base around the glycosyl bond[10, 11] leading to two conformers: *syn* and *anti*.

The conformational analysis of the furanose ring follows the concept of pseudorotation introduced by Altona and Sundaralingam[7, 8]. According to this description, each conformation of the furanose ring is determined by two parameters, the phase angle of pseudorotation, P, and the degree of puckering, τ_m. The ribose conformations are divided into two types: type N, where P varies between -90° and $+90^\circ$, which includes the classical C(3′)-*endo* conformation; type S, where P is comprised between $+90^\circ$ and 270°, which includes the classical

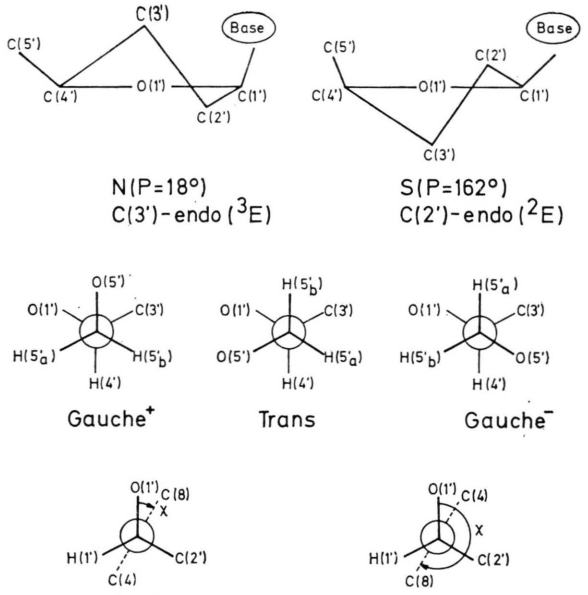

Fig. 1. Schematic representation of the major mode of internal motions in purine(β)ribosides. The definition of the glycosyl torsion angle follows Sundaralingam[11].

C(2′)-*endo* conformation. In the solid state, the β sugars present two narrow ranges for P, 3° to 23° for the type N and 139° to 175° for the type S conformation, and the degree of pucker, τ_m, is relatively constant around 38°. The vicinal ribose proton coupling constants (J) can be calculated for various values of P at fixed τ_m. With these values, it is possible to obtain the mole fractions of the conformers N and S from

$$J^{ij}_{obs} = [N] J^{ij}_N + [S] J^{ij}_S ,$$

since in solution both conformers exist in a dynamic equilibrium, $S \rightleftarrows N$, rapid on the time-scale of a high resolution NMR experiment[9]. The theoretical values for the vicinal ribose proton coupling constants in the N and S states were determined from crystal structure data and on the basis of the following Karplus equation, obtained from results on the common purine(β)ribosides in ND_3 [4],

$$J^{ij} = 10.0 \cos^2 \varphi^{ij} - 0.90 \cos \varphi^{ij} .$$

The parameters entering this Karplus equation are determined on the assumption that the coupling constant $J^{2'3'}$ as well as the sum $J^{1'2'} + J^{3'4'}$ (Σ) are nearly independent of the position of the $S \rightleftarrows N$ equilibrium. The necessary torsion angles are taken from crystal data and are those of the ribose conformers occupying the centers of the pseudorotational ranges observed in the solid state[7]. We have observed that the values of $J^{2'3'}$ and Σ, though nearly constant, vary with the solvent. In ND_3, their values are respectively 5.0 and 9.4 Hz and, in D_2O, they are equal to 5.3 and 9.8 Hz. The Karplus parameters in D_2O are therefore 10.5 and −0.86 Hz instead of 10.0 and −0.90 Hz obtained for ND_3 solutions. Because of the numerous assumptions underlying the pseudorotation model, we have used throughout the Karplus equation given above. A better fit to the observed coupling constants in D_2O would be obtained with the other parameters. However, next to small changes in the phase angles of pseudorotation, the differences between the calculated populations of the conformers do not exceed 5%.

The three staggered classical conformations of the exocyclic group CH_2OD are referred to as *gauche*$^+$ (g^+, *gauche-gauche*), *trans* (t, *gauche-trans*), and *gauche*$^-$ (g^-, *trans-gauche*). The mole fractions of the different rotamers were obtained from:

$$P_{g^+} = 1.46 - (J^{4'5'_a} + J^{4'5'_b})/8.9 ,$$

$$P_t = J^{4'5'_a}/8.9 - 0.23 ,$$

$$P_{g^-} = J^{4'5'_b}/8.9 - 0.23 .$$

The equations were obtained with the given Karplus equation following the procedure of Blackburn *et al.*[12], and Hruska *et al.*[13]. The rotamers t and g^- are not unequivocally assigned. Our choice

$$\delta(H5'_a) > \delta(H5'_b) ; \quad J^{4'5'_a} > J^{4'5'_b}$$

is such that in the common nucleosides dissolved in ND_3 the population of the g^- rotamer is at low temperature minimal. This yields relative concentrations of the conformers in agreement with solid state data[24]. This assignment is the same as that proposed by Remin and Shugar[14]. However, on the basis of this comparison with solid state, the assignment is reverse for nucleosides in aqueous solutions, since in such solutions one has

$$\delta(H5'_a) > \delta(H5'_b) ; \quad J^{4'5'_a} < J^{4'5'_b} .$$

This assignment is opposite to that of Remin and Shugar[14] and in agreement with that suggested for aqueous solutions of guanosine phosphates[15]. This solvent dependence of the specific assignment of the protons $H5'_a$ and $H5'_b$ could mean either that the shifts of both protons are solvent dependent or that in ND_3 at low temperature the g^- rotamer is more stable than the t rotamer.

4. Results

4.1. Chemical shifts

The chemical shifts of 2-azaadenosine, formycin, 8-bromoinosine, and 8-bromoxanthosine dissolved in ND_3 and observed at two temperatures are collected in Table I. This table contains also the chemical shifts of formycin, formycin B, nebularine, and 8-bromoinosine dissolved in D_2O and observed at two temperatures. The chemical shifts of formycin B and nebularine dissolved in ND_3 were previously published[4]. We have also analysed formycin and 8-bromoadenosine in pyridine solutions. The chemical shifts can be found in Table I.

4.2. Coupling constants

The coupling constants of 2-azaadenosine, formycin, 8-bromoinosine, and 8-bromoxanthosine dissolved in ND_3 are contained in Table II. In this table are also collected the coupling constants of formycin, formycin B, nebularine, and 8-bromoinosine in D_2O. In Figs 2 to 5 the experimental and

Table I. Chemical shifts in ppm at two different temperatures for 2-azaadenosine (2-aA), formycin (Fo), 8-bromoinosine (8-BrI), and 8-bromoxanthosine (8-BrX) dissolved in ND_3; for formycin, formycin B (FoB), 8-bromoinosine, and nebularine (PR) dissolved in D_2O; for formycin and 8-bromoadenosine (8-BrA) dissolved in pyridine.

ND_3	2-aA		Fo		8-BrI		8-BrX	
δ °C	+40	−60	+40	−60	+40	−60	+40	−60
1′	5.75	5.79	4.80	4.83	5.52	5.57	5.34	5.38
2′	4.18	4.21	4.21	4.20	4.74	4.78	4.71	4.71
3′	3.84	3.86	3.84	3.89	3.88	3.86	3.81	3.77
4′	3.67	3.73	3.60	3.61	3.68	3.74	3.58	3.63
$5'_a$	3.29	3.43	3.38	3.41	3.39	3.42	3.34	3.36
$5'_b$	3.26	3.29	3.23	3.24	3.24	3.28	3.17	3.18

D_2O	Fo		FoB		8-BrI		PR	
δ °C	+60	+10	+60	+10	+60	+10	+60	+10
1′	5.21	5.17	5.25	5.21	6.06	6.09	6.13	6.10
2′	4.59	4.57	4.60	4.57	5.03	5.07	4.78	4.75
3′	4.32	4.35	4.36	4.37	4.46	4.48	4.40	4.39
4′	4.18	4.22	4.22	4.25	4.19	4.23	4.22	4.22
$5'_a$	3.88	3.91	3.92	3.94	3.86	3.88	3.86	3.86
$5'_b$	3.76	3.79	3.81	3.83	3.80	3.83	3.79	3.79

Pyr	Fo	8-BrA
δ °C	+40	+40
1′	6.05	6.65
2′	5.55	5.88
3′	5.06	5.08
4′	4.79	4.76
$5'_a$	4.37	4.29
$5'_b$	4.12	4.10

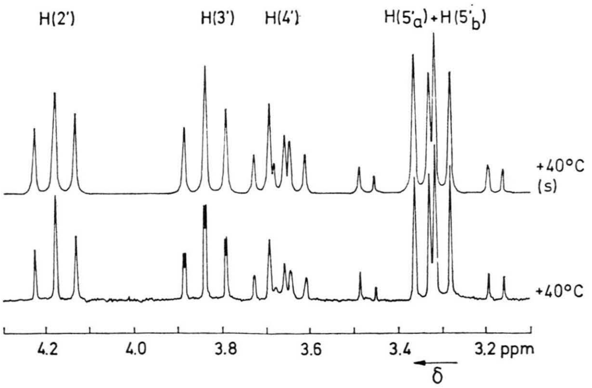

Fig. 2. Experimental proton high resolution spectrum of a solution of 2-azaadenosine in ND_3 at +40 °C covering the region of the protons $H(2')$ to $H(5'_a)$ and $H(5'_b)$ compared with the simulated spectrum.

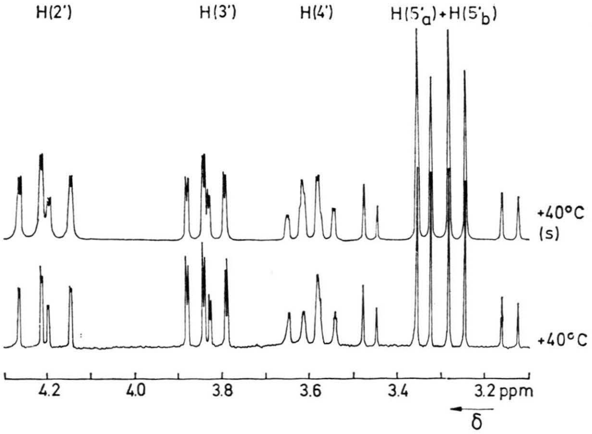

Fig. 3. Experimental proton high resolution spectrum of a solution of formycin in ND_3 at 40 °C covering the region of the protons $H(2')$ to $H(5'_a)$ and $H(5'_b)$ compared with the simulated spectrum.

Table II. Coupling constants in Hz at two different temperatures for 2-azaadenosine (2-aA), formycin (Fo), 8-bromoinosine (8-BrI), and 8-bromoxanthosine (8-BrX) dissolved in ND_3; for formycin, formycin B (FoB), 8-bromoinosine, and nebularine (PR) dissolved in D_2O; for formycin and 8-bromoadenosine (8-BrA) dissolved in pyridine.

ND_3	2-aA		Fo		8-BrI		8-BrX	
J °C	+40	−60	+40	−60	+40	−60	+40	−60
1′2′	4.6	4.7	6.7	7.0	7.2	7.8	7.2	7.8
2′3′	5.0	4.6	5.0	4.9	5.2	4.9	5.3	4.9
3′4′	4.7	4.5	3.9	3.0	2.0	0.9	1.8	0.8
$4'5'_a$	3.7	4.1	3.2	3.0	3.1	3.9	2.7	3.1
$4'5'_b$	3.5	3.1	3.8	3.3	3.4	3.4	3.3	3.3
$5'_a5'_b$	−12.2	−12.2	−12.2	−12.3	−12.4	−12.4	−12.5	−12.5

D_2O	Fo		FoB		8-BrI		PR	
J °C	+60	+10	+60	+10	+60	+10	+60	+10
1′2′	7.0	7.4	7.1	7.4	6.3	6.6	5.4	5.7
2′3′	5.6	5.5	5.4	5.5	5.7	5.4	5.4	5.2
3′4′	3.6	2.8	4.1	3.2	3.5	2.4	4.2	3.8
$4'5'_a$	3.3	3.0	3.0	3.5	3.1	2.7	3.2	3.0
$4'5'_b$	4.1	3.9	4.3	3.7	4.5	4.4	4.4	3.8
$5'_a5'_b$	−12.7	−12.8	−12.6	−12.8	−12.5	−12.5	−12.7	−12.7

Pyr	Fo	8-BrA
J °C	+40	+40
1′2′	7.4	6.9
2′3′	5.0	5.1
3′4′	2.3	2.2
$4'5'_a$	2.5	2.7
$4'5'_b$	2.4	2.9
$5'_a5'_b$	−12.3	−12.3

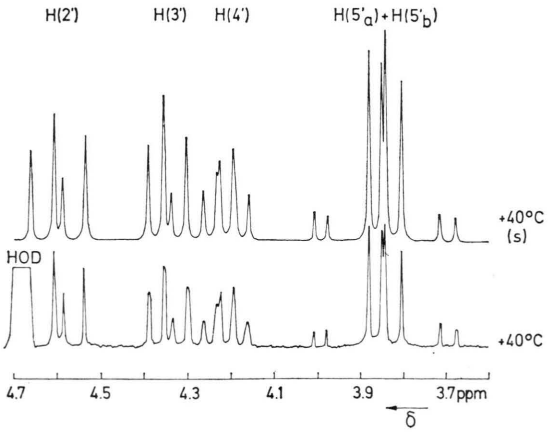

Fig. 4. Experimental proton high resolution spectrum of a solution of formycin in D_2O at 40 °C covering the region of the protons H(2′) to $H(5'_a)$ and $H(5'_b)$ compared with the simulated spectrum.

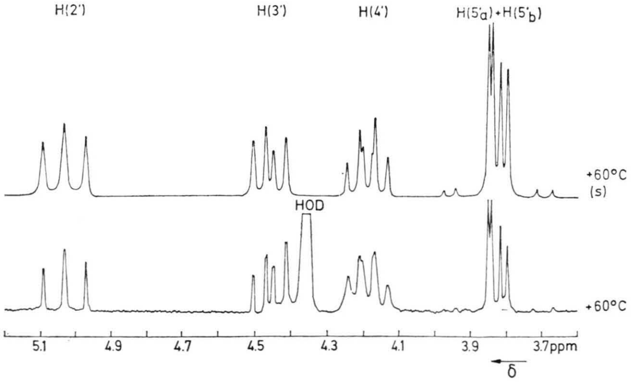

Fig. 5. Experimental proton high resolution spectrum of a solution of 8-bromoinosine in D_2O at +60 °C covering the region of the protons H(2′) to $H(5'_a)$ and $H(5'_b)$ compared with the simulated spectrum.

Table III. Results of the conformational analysis for the compounds studied.

ND_3	T[°C]	P_N	[N]	P_S	[S]	[g^+]	[t]
2-aA	+40	10	.49	175	.51	.65	.19
	−60	10	.47	175	.53	.65	.23
Fo	+40	10	.31	145	.69	.67	.13
	−60	3	.28	145	.72	.75	.11
8-BrI	+40	25	.20	175	.80	.73	.12
	−60	10	.12	175	.88	.64	.21
8-BrX	+40	25	.20	175	.80	.79	.07
	−60	10	.12	175	.88	.74	.12
D_2O							
Fo	+60	25	.28	145	.72	.63	.23
	+10	10	.24	145	.76	.68	.21
FoB	+60	25	.28	145	.72	.64	.25
	+10	25	.24	145	.76	.65	.19
8-BrI	+60	25	.35	161	.65	.61	.27
	+10	3	.26	175	.74	.67	.27
PR	+60	10	.43	161	.57	.61	.26
	+10	3	.40	161	.60	.69	.20
8-BrA	+30	3	.25	161	.75	.83	.12

taken from ref. 29.

simulated spectra of 2-azaadenosine in ND_3, formycin in ND_3 and D_2O, and 8-bromoinosine in D_2O are presented. The spectra simulation was done with the values of the chemical shifts and of the coupling constants contained in Tables I and II.

The mole fractions of the S and N states of the ribose and of the two rotamers of the exocyclic group g^+ and t are presented in Table III.

2-Azaadenosine in ND_3

The substitution of the C(2) carbon by a nitrogen introduces only slight changes in the mole fractions of the different conformers when compared with those of adenosine [19]: a small decrease in the populations of the S state and of the g^+ rotamer. Therefore, the preference for the S state is not as marked as in adenosine, where the mole fraction is around 0.60. In 2-azaadenosine, both states of the ribose are roughly equally populated. However, the rotamer g^+ still dominates with a mole fraction around 0.65. It should be remembered that the substitution of the C(8) carbon by a nitrogen introduces more pronounced changes in the ribose conformers, especially in those of the exocyclic group [5, 16].

Nebularine in D_2O

Nebularine, or purine(β)riboside (PR), was already analysed in ND_3 solutions [4]. The conformational behaviour of nebularine dissolved in ND_3 was found to be very similar to that of the common purine(β)ribosides. The same is valid for D_2O solutions. The mole fractions of the different conformers of nebularine dissolved in D_2O compare well with those of inosine or adenosine dissolved in D_2O (see, for example, [5]).

Formycin in ND_3

We have already presented the results for formycin B, a structural analog of inosine, dissolved in ND_3 [4]. We present now the results for formycin, the equivalent structural analog of adenosine. These two compounds have very similar conformational parameters with strong differences from the common purine(β)ribosides. A significant increase in the population of the S state at all temperatures occurs together with a slight increase in the population of the g^+ rotamer at low temperature. The conformational characteristics of the C-nucleosides formycin and formycin B bear on the other hand a close similarity to those of the 8-bromopurineribosides. This will be shown below, but we will first consider the results for formycin and formycin B dissolved in D_2O.

Formycin and formycin B in D_2O

As in ND_3 solutions, formycin and formycin B in D_2O solutions differ from adenosine and inosine because of their increased populations of the S state of the ribose. However, this increase in the mole fraction of the S state is not followed by an increase of the g^+ rotamer as could be observed in ND_3 solutions. The population of the g^+ rotamer of formycin and formycin B in D_2O has even decreased compared to that of adenosine and inosine.

8-Bromoinosine and 8-bromoxanthosine

Because of the observed similarity between the C-nucleosides and the 8-bromopurineribosides, we analysed in addition to 8-BrA and 8-BrG previously studied[4] two other 8-bromopurineribosides. The main difference between the common and the 8-bromopurineribosides occurs in the mole fraction of the S state of the ribose which, at $-60\ ^\circ C$, is near to 0.90. However, the populations of the g^+ rotamer of the exocyclic group are lower in the 8-bromopurineribosides than in the corresponding common purineribosides. Also, the population of the g^+ rotamer decreases with decreasing temperature. Such a behaviour of the exocyclic group has only be observed with the 8-azapurineribosides in ND_3 [5]. The last effect was more pronounced in case of the 8-azapurineribosides and is not present in D_2O solutions where the population of the g^+ rotamer does not vary much with temperature. This difference between ND_3 and D_2O solutions occurs also with 8-bromoinosine. For 8-bromoinosine dissolved in D_2O, the population of the g^+ rotamer at low temperature is lower than that in inosine dissolved in D_2O but increases with decreasing temperature. It should also be added that, for 8-bromoinosine dissolved in D_2O, the increase in the mole fraction of the S state is only marked at low temperature. Concerning the populations of the state of the ribose, the 8-bromopurineribosides are very different from the 8-azapurineribosides. Indeed, the population of the S state of the 8-bromopurineribosides is always greater than in the common purineribosides, independently of the solvent, while, for the 8-azapurineribosides, the S state dominates with a mole fraction of 0.60 in ND_3 and both states are roughly equally populated in D_2O solutions.

Pyridine solutions

Table IV contains the results of the theoretical analysis for formycin and 8-bromoadenosine. For comparison, the results for adenosine and 8-azaadenosine, taken from reference[5], are also given in Table IV.

When dissolved in pyridine, adenosine, 8-azaadenosine, formycin and 8-bromoadenosine all behave similarly. The S state of the ribose dominates with a population larger than 0.60 for adenosine and 8-azaadenosine and around 0.80 for formycin and 8-bromoadenosine. The mole fraction of the g^+ rotamer is to a large extent the main conformer of the exocyclic group. Thus, in pyridine solutions, the similarity between 8-bromoadenosine and formycin is complete. Also, 8-azaadenosine differs from adenosine and from 8-bromoadenosine and formycin.

5. Discussion and Conclusions

5.1. *Conformations of purine(β)riboside analogs in solution*

It is possible to divide the purineriboside analogs we have studied into three classes. The first group would contain the analogs with essentially the same conformational behaviour as the common purine(β) ribosides; it includes tubercidin, nebularine, and 2-azaadenosine. One may also add 6-thiopurineriboside[17]. In this group, independently of the solvent, the S state of the ribose dominates (0.55 –

Table IV. Results of the conformational analysis for 8-bromoadenosine and formycin dissolved in pyridine. For comparison, the results for 8-azaadenosine (8-aA) and adenosine (A) dissolved in pyridine are also given[5].

Pyr	T[°C]	P_N	[N]	P_S	[S]	[g^+]	[t]
8-BrA	+40	10	.23	175	.77	.83	.07
Fo	+40	10	.22	161	.78	.91	.05
8-aA	+40	10	.38	175	.62	.60	.16
A	+40	3	.34	175	.66	.87	.07

0.65) and the exocyclic group is to a large extent in the g^+ conformer (0.65 – 0.75). The second group would contain the 8-bromopurineribosides with the C-nucleosides formycin and formycin B. This group is characterized by a strong preference for the S state of the ribose (0.70 – 0.80) and, especially in ND_3 or pyridine solutions, by a predominance of the g^+ rotamer of the exocyclic group (0.70 – 0.90). The third group would comprise the 8-azapurineribosides. The conformational properties of these analogs are strongly dependent on the solvent. In D_2O solutions, the 8-azapurineribosides are characterized by equal populations of the S and N states and by a smaller preference for the g^+ rotamer (0.55) than the other purineribosides. The destabilization of the g^+ rotamer is observed in all solvents studied.

In a previous paper [4], it was derived that the g^+ rotamer of the exocyclic group is correlated with the S state of the ribose in purine(β)ribosides. A comparison between the different conformer populations in the three main groups of compounds studied shows that this correlation holds for the purine riboside analogs. In aqueous solutions of pyrimidine nucleosides, Hruska [9] has correlated the g^+ conformation of the hydroxymethyl group with the N state of the ribose. Both correlations are in agreement with molecular orbital calculations [18]. According to these calculations, the S state of the ribose in purine nucleosides should behave similarly to the N state of the ribose in pyrimidine nucleosides with respect to the stabilization of the g^+ rotamer.

We have not yet discussed the third major mode of internal motion of the purine nucleosides, the rotation about the glycosidic bond. The glycosyl torsion angles are commonly distributed in two ranges called *anti* and *syn* [10, 11]. In the *anti* range, the C(8) atom is on the same hemisphere as O(1′) when viewed down along the C(1′) – N(9) bond in a Newman projection. In the *syn* range, C(8) and O(1′) are in opposite hemispheres. From nuclear Overhauser enhancements and proton relaxation measurements, it has been shown that in solution there exists a dynamic equilibrium between the *syn* and *anti* conformations of the purine(β)-ribosides [6, 19, 20–23]. For the purine(β)ribosides, we have further correlated the N state of the ribose with the *anti* conformation of the base and the S state with the *syn* conformation. These correlations stimulated by solid state data [24] allowed to rationalize both the NOE enhancements and the T_1 measurements [6, 19]. The correlation between the N state and the *anti* conformation is certainly strong while the S state of the ribose should equally favor the *syn* and *anti* conformations [25–28]. It has recently been shown that 3′-aminopurine(β)-riboside, where the ribose is up to 90% in the N state, is exclusively in the *anti* conformation [29]. It has also been shown that, when the base is restricted to the *syn* range as in the 8-bromopurineribosides, the S state of the ribose is strongly favored [4, 29]. With the S state of the ribose, the base should occur in a $\approx 1:1$ equilibrium mixture of the *syn* and *anti* conformations. This is in agreement with previous speculations [7] and with thermodynamic quantities derived from the temperature dependence of the S/N ratio [4].

On the basis of the *syn* $\rightleftarrows$ *anti* equilibrium in the common purineribosides and of the *syn* conformation of the 8-bromopurineribosides, it is possible to draw conclusions on the preferred glycosyl torsion angle ranges of the C-nucleosides and of the 8-azapurineribosides. From the strong similar conformational behaviour of the 8-bromopurineribosides and the C-nucleosides, we would conclude that formycin and formycin B are also restricted to the *syn* range. On the other hand, since the conformational behaviour of the 8-azapurineribosides is opposite to that of the 8-bromopurineribosides, it is tempting to conclude that in dissolved 8-azapurineribosides the *syn* $\rightleftarrows$ *anti* equilibrium is shifted toward the *anti* range. The comparison with the 6-azapyrimidine derivatives supports this conclusion, since these compounds are known from X-ray and NMR analyses to prefer the *anti* range with the rotamers *t* and g^- [30, 31]. The destabilization of the g^+ rotamer could therefore be due to electrostatic repulsion between the aza part of the base and O(5′) of the exocyclic group.

5.2. Comparison with crystal structure data

It is interesting, at this point, to compare the populations of the various conformers in solution with the main conformations observed in crystals of purineriboside analogs [31, 32]. The common purineribosides show a definite preference for the family of conformations *anti*-S-g^+ [24]. The N state of the ribose is only found with the *anti* conformation. When the sugar pucker is S, both the *syn* and *anti* conformations of the base are found with equal frequencies [24]. Further, the *syn*-S conformation is always linked with the g^+ rotamer. In the solid state, nebularine, 2-azaadenosine, and tubercidin exhibit conformations similar to adenosine [32]. In a review on the structure and conformation of nucleoside analogs in the solid state, Sundaralingam [32] could conclude that the *syn* conformation is favored for the analogs containing the C-glycosyl bond and that the 8-azapurines show persistently higher *anti* glycosyl angles than the common substrates.

These conclusions are exemplified in Table V where we have collected the frequencies of occurence of the various conformers. The populations of the ribose conformers of the common purine-ribosides in the solid state compare well with those measured in solution. It can also be observed that the mole fractions of 2-azaadenosine, nebularine, 6-CH_3-9-β-D-ribofuranosylpurine, and the 6-thiopurineribosides are not significantly different from those of adenosine, inosine, guanosine, and xanthosine. The 8-bromopurineribosides were exclusively found in the *syn*-S-g^+ conformation. This is paralleled by the definite preference for the S-g^+ conformation of these substances in solution [4, 40]. The C-nucleosides present a net decrease in the *anti* population and in the g^+ rotamer. These compounds are the only ones showing the conformation families *syn*-S-*t* and *syn*-N. The preference of the C-nucleosides for the *syn* range would corroborate our speculations about their *syn* conformation in solution which were based on their resemblance with the 8-bromopurineribosides. However, in the solid state, they do not show as strong a preference for the g^+ rotamer as in solution. Only one crystal structure of 8-azapurineribosides is known, which makes the comparison fallacious. The conformation of 8-azaadenosine is found to be similar to that of formycin in formycin·H_2O crystals [31]. This resemblance could be accidental, for, in solution, both kinds of compounds differ greatly in their conformational behaviour. The observed conformations of the 8-azapurineribosides depend strongly on the solvent. It is thus probable that, in the solid state, packing effects or molecules of crystallization could influence the molecular conformation adopted by the 8-azapurine(β) ribosides.

Table V. Frequencies of observation of the three major modes of internal motion of purine(β) nucleosides and analogs in the solid state.

	[*anti*]	[S]	[g^+]	Number of conformations
Common nucleosides [a]	0.67	0.67	0.78	9
PR [b], 2-aA [c], T [d], 6-CH_3-PR [e], 6-S-I [f], 6-S-G [g]	0.71	0.71	0.71	7
8-BrA [h], 8-BrG [i]	0.0	1.0	1.0	2
C-nucleosides [j]	0.20 [k]	0.60	0.60	5

[a] The common nucleosides comprise adenosine (T. F. Lai and R. E. Marsh, Acta Crystallogr. **B 28,** 1982 [1972]; A. E. V. Haschemeyer and H. M. Sobell, ibid. **18,** 525 [1965]; K. Shikata, T. Ueki, and T. Mitsui, ibid. **B 29,** 31 [1973]), guanosine and inosine (U. Thewalt, C. E. Bugg, and R. E. Marsh, Acta Crystallogr. **B 26,** 1089 [1970]; A. R. I. Munns and P. Tollin, ibid. **B 26,** 1101 [1970]), and xanthosine (G. Koyama, H. Nakamura, and H. Umezawa, Acta Crystallogr. **B 32,** 969 [1976]).

[b] Nebularine or 9-β-D-ribofuranosylpurine (T. Takeda, Y. Ohashi, and Y. Sasada, Acta Crystallogr. **B 30,** 825 [1974]).

[c] 2-Azaadenosine (ref. 31).

[d] Tubercidin (J. Abola and M. Sundaralingam, Acta Crystallogr. **B 29,** 697 [1973]; R. M. Stroud, Acta Crystallogr. **B 29,** 690 [1973]).

[e] 6-Methyl-9-β-D-ribofuranosylpurine (T. Takeda, Y. Ohashi, and Y. Sasada, Acta Crystallogr. **B 31,** 1202 [1975]).

[f] 6-Thio-inosine (E. Shefter, J. Pharm. Sci. **57,** 1157 [1968]).

[g] 6-Thio-guanosine (U. Thewalt and C. E. Bugg, J. Amer. Chem. Soc. **94,** 8892 [1972]).

[h] 8-Bromoadenosine (ref. 26).

[i] 8-Bromoguanosine (C. E. Bugg and U. Thewalt, Biochem. Biophys. Res. Common. **37,** 623 [1969]).

[j] The C-nucleosides comprise formycin (ref. 38 and 39), 2-methylformycin (J. Abola, M. J. Sims, D. J. Abraham, A. F. Lewis, and L. B. Townsend, J. Med. Chem. **17,** 62 [1974]), formycin B and oxoformycin B (G. Koyama, H. Nakamura, H. Umezawa, and Y. Iitaka, Acta Crystallogr. **B 32,** 813 [1976]).

[k] This value is obtained when one considers the so-called "*high-anti*" region as belonging to the *syn* range. Otherwise, this value would be 0.40 ("*high-anti*" ≡ *anti*).

5.3. *The intramolecular hydrogen bond O(5')-H . . . N(3)*

The main difference between pyrimidine(β)-nucleosides and purine(β) nucleosides lies in the preferred *anti* range of the former [11, 24]. The occurence of the purine(β) nucleosides in both the *anti* and *syn* ranges with roughly equal frequencies was attributed to the stabilization of the *syn* conformation through a hydrogen bond between the exocyclic group and the base O(5')-H . . . N(3) [11]. In fact, in the solid state, the *syn*-S-g^+ conformation of purine(β) ribosides is invariably involved in this intramolecular hydrogen bond [24]. Further, considering only the common purine(β) ribosides, the *syn* range is always associated with the S-g^+ conformation of the ribose.

These findings are corroborated by theoretical calculations which show that, in the absence of an intramolecular hydrogen bond, there is a definite preference of the nucleosides for the *anti* conformation[33]. However, when the calculations take into account the possibility of an intramolecular hydrogen bond in the g^+ conformation, the *syn*-S conformation is strongly preferred, while the N state of the ribose is disfavored in the *anti* and *syn* ranges[33, 34]. Such a stabilization of the *syn* conformation is also calculated in formycin[34].

The presence of an intramolecular hydrogen bond between O(5′) and N(3) in aqueous solution of 8-bromoadenosine was postulated from the strong preference of the g^+ rotamer[40]. By comparison with the isopropylidene derivatives where such a bond may not form, we also concluded that the high population of the g^+ rotamer is due to an intramolecular hydrogen bond[4]. On the basis of NOE measurements, the higher *syn* population observed in adenosine compared to 3-deazaadenosine was also attributed to the formation of such a hydrogen bond[35]. Ultrasonic relaxation measurements did not contradict the possibility of an intramolecular hydrogen bond[36]. We have studied[37] 5′-deoxyadenosine where the replacement of the 5′-hydroxyl group by a hydrogen prevents the formation of a hydrogen bond between the exocyclic group and the base. This replacement brings an interchange in the relative populations of the S and N states of the ribose[37]. Indeed, in ND_3 and pyridine solutions at 40 °C, the N state dominates with a mole fraction equal to 0.60 and, in D_2O solution, both states are equally populated[37]. This can be taken as a further argument in favor of the existence of a hydrogen bond between N(3) and O(5′).

All these observations strengthen the previous conjectures about the existence of an intramolecular hydrogen bond in solution of purine(β)ribosides. The strong preference for the S-g^+ conformation of the 8-bromopurineribosides and C-nucleosides in solution indicates that such an intramolecular bond stabilizes the *syn* conformation in these two kinds of compounds. From the graphs of Fig. 6, it can be seen that a hydrogen bond between O(5′) and N(3) can only be formed in narrow regions of the *syn* range with the exocyclic group in g^+. In the so-called "*high-anti*" conformation found in crystals of formycin·H_2O[38] this H-bond may not form, thereby excluding the "*high-anti*" conformation of formycin in solution. Incidentally it can be observed that with the *t* rotamer a very narrow region

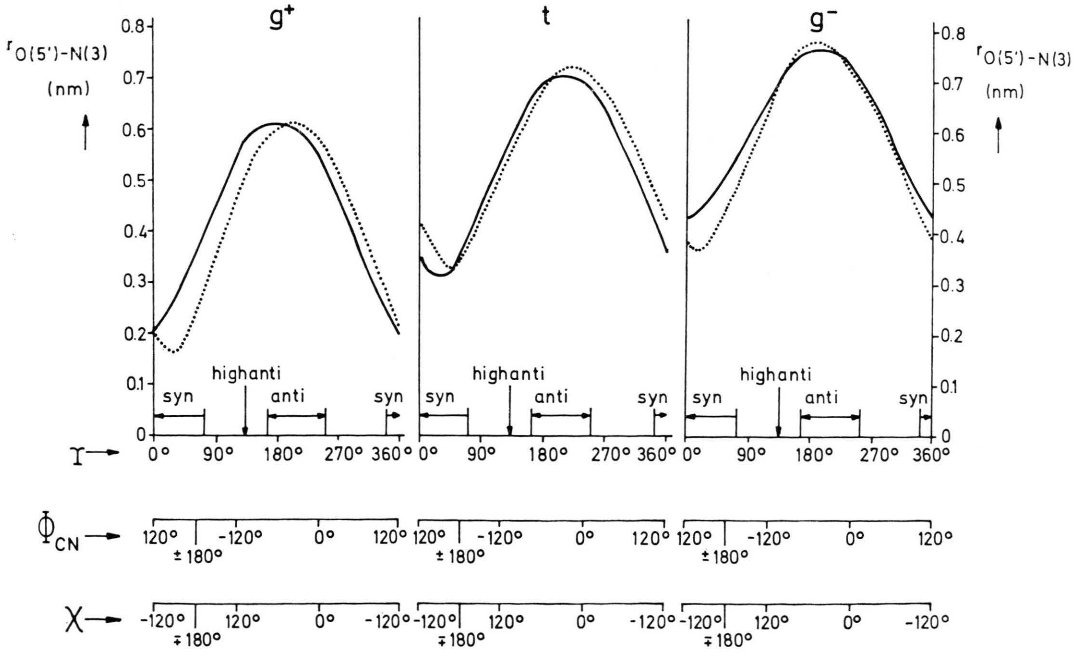

Fig. 6. Distance between O(5′) and N(3) in formycin for the three rotamers of the exocyclic group and the two states of the ribose as function of the glycosyl torsion angle.

of the *syn* range allows the formation of such a H-bond. This has been observed in crystals of formycin·HBr[39]. This interaction could also be a stabilization factor for the *t* rotamer.

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[1] R. J. Suhadolnik, Nucleoside Antibiotics, Wiley-Interscience, New York 1970.

[2] Chemistry, Biology, and Clinical Uses of Nucleoside Analogs, Ann. N. Y. Acad. Sci., **Vol. 255** (A. Bloch, ed.), New York 1975.

[3] A. Bloch, Ann. N. Y. Acad. Sci. **255,** 576 [1975].

[4] E. Westhof, O. Röder, I. Croneiss, and H.-D. Lüdemann, Z. Naturforsch. **30 c,** 131 [1975].

[5] H.-D. Lüdemann, E. Westhof, and I. Cuno, Z. Naturforsch. **31 c,** 135 [1976].

[6] H.-D. Lüdemann, E. Westhof, and O. Röder, Europ. J. Biochem. **49,** 143 [1974].

[7] C. Altona and M. Sundaralingam, J. Amer. Chem. Soc. **95,** 2333 [1973].

[8] C. Altona and M. Sundaralingam, J. Amer. Chem. Soc. **94,** 8205 [1972].

[9] F. E. Hruska, The Jerusalem Symposia on Quantum Chemistry and Biochemistry, **Vol. V,** p. 345 (E. D. Bergmann and B. Pullman, eds.), 1973.

[10] J. Donohue and K. N. Trueblood, J. Mol. Biol. **2,** 363 [1960].

[11] M. Sundaralingam, Biopolymers **7,** 821 [1969].

[12] B. J. Blackburn, A. A. Grey, I. C. P. Smith, and F. E. Hruska, Can. J. Chem. **48,** 2866 [1970].

[13] F. E. Hruska, D. J. Wood, R. J. Mynott, and R. H. Sarma, Fed. Eur. Biochem. Soc. Lett. **31,** 153 [1973].

[14] M. Remin and D. Shugar, Biochem. Biophys. Res. Commun. **48,** 636 [1972].

[15] S. Tran-Dinh and W. Guschlbauer, Nucleic Acids Res. **2,** 873 [1975].

[16] C. H. Lee, F. E. Evans, and R. H. Sarma, J. Biol. Chem. **250,** 1290 [1975].

[17] F. E. Evans and R. H. Sarma, J. Amer. Chem. Soc. **97,** 3215 [1975].

[18] A. Saran, B. Pullman, and D. Perahia, Biochim. Biophys. Acta **287,** 211 [1972].

[19] H.-D. Lüdemann, O. Röder, E. Westhof, E. v. Goldammer, and A. Müller, Biophys. Struct. Mechanism **1,** 121 [1975].

[20] P. Hart and J. P. Davis, J. Amer. Chem. Soc. **93,** 753 [1971].

[21] R. Schirmer, J. P. Davis, J. Noggle, and P. Hart, ibid. **94,** 3266 [1972].

[22] S. Tran-Dinh, W. Guschlbauer, and M. Gueron, ibid. **94,** 7903 [1972].

[23] M. Gueron, C. Chachaty, and S. Tran-Dinh, Ann. N. Y. Acad. Sc. **222,** 307 [1973].

[24] M. Sundaralingam, The Jerusalem Symposia on Quantum Chemistry and Biochemistry, **Vol. V,** p. 417 (E. D. Bergmann and B. Pullman, eds.), 1973.

[25] S. T. Rao and M. Sundaralingam, J. Amer. Chem. Soc. **92,** 4963 [1970].

[26] S. S. Tavale and H. M. Sobell, J. Mol. Biol. **48,** 109 [1970].

[27] D. W. Miles, L. B. Townsend, M. J. Robins, R. K. Robins, W. H. Inskeep, and H. Eyring, J. Amer. Chem. Soc. **93,** 1600 [1971].

[28] A. V. E. Haschemeyer and A. Rich, J. Mol. Biol. **27,** 369 [1967].

[29] H. Plach, I. Cuno, and H.-D. Lüdemann, Jahrestagung der Dt. Ges. f. Biophysik, p. 36, Regensburg 1976.

[30] D. J. Wood, F. E. Hruska, R. J. Mynott, and R. H. Sarma, Can. J. Chem. **51,** 2571 [1973].

[31] D. J. Hodgson and P. Singh, Environmental Effects on Molecular Structure and Properties, p. 343 (B. Pullman, ed.), 1976.

[32] M. Sundaralingam, Ann. N. Y. Acad. Sci. **255,** 3 [1975].

[33] H. Berthod and B. Pullman, Biochim. Biophys. Acta **232,** 595 [1971].

[34] M. Knappenberg and J. Dirkx, Arch. Internat. Physiol. Biochem. **82,** 777 [1974].

[35] S. Kitano, Y. Mizuno, M. Ueyama, K. Tori, M. Kamisaku, and K. Ajisaka, Biochem. Biophys. Res. Commun. **64,** 996 [1975].

[36] P. R. Hemmes, L. Oppenheimer, and F. Jordan, J. Amer. Chem. Soc. **96,** 6023 [1974].

[37] H.-D. Lüdemann, E. Westhof, I. Cuno, and A. Müller-Broich, VIIth International Conference on Magnetic Resonance in Biological Systems (St. Jovite, Canada, 1976).

[38] P. Prusiner, T. Brennan, and M. Sundaralingam, Biochemistry **12,** 1196 [1973].

[39] G. Koyama, H. Umezawa, and Y. Iitaka, Acta Crystallogr. **B 30,** 1511 [1974].

[40] R. H. Sarma, C.-H. Lee, F. E. Evans, N. Yathindra, and M. Sundaralingam, J. Amer. Chem. Soc. **96,** 7337 [1974].